CERTIFICATION UNDER 37 CFR 1.10

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Patti Selan

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors:	Lam, J. et al.	Examiner:
Serial No:	to be assigned	Art Unit:
Filed:	February 9, 2001 (herewith)	
Title:	NUCLEIC ACIDS LABELLED WITH EXTENDED RHODAMINE DYES	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D. C. 20231

Sir:

Applicants submit the Preliminary Amendment concurrently with this application. Prior to examination on the merits, please amend the application as follows:

This is a preliminary amendment under 37 CFR 1.53(b) for a divisional application of pending application Serial No. 09/325,423, filed on June 3, 1999.

I. AMENDMENTS

Please enter the following amendments to the above-identified patent application. No new matter has been added to the application.

In the specification:

At page 1, top, delete " EXTENDED RHODAMINE COMPOUNDS USEFUL AS FLUORESCENT LABELS", and insert therefor -- NUCLEIC ACIDS LABELLED WITH EXTENDED RHODAMINE DYES --

At page 1, after the title, please insert: --This application is a division of Application No. 09/325,243, filed June 3, 1999, which is incorporated herein by reference.--

In the claims:

Please add new claims 46-75.

Please cancel claims 1-45 without prejudice.

-- 46. A labelled nucleic acid compound having the formula:

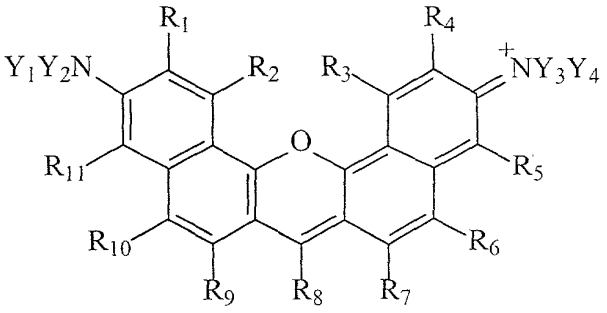
NUC—L—D

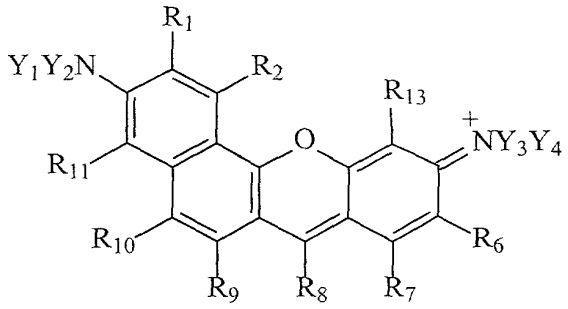
wherein

NUC is a nucleic acid compound selected from a nucleoside, a nucleotide, a polynucleotide and analogs thereof;

L is a linkage; wherein if NUC comprises a purine base, the linkage is attached to the 8-position of the purine, if NUC comprises a 7-deazapurine base, the linkage is attached to the 7-position of the 7-deazapurine, and if NUC comprises a pyrimidine base, the linkage is attached to the 5-position of the pyrimidine; and

D is an extended rhodamine dye comprising the structures:





wherein

R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_9, R_{10}, R_{11}, and R_{13} when taken alone are selected from –H, alkyl, alkyl independently substituted with one or more Z_1, heteroalkyl, heteroalkyl independently substituted with one or more Z_1, aryl, aryl independently substituted with one or more Z_1, heteroaryl, heteroaryl independently substituted with one or more Z_1, arylalkyl, arylalkyl independently substituted with one or more Z_1, heteroarylalkyl, heteroarylalkyl independently substituted with one or more Z_1, halogen, $-OS(O)_2OR$, $-S(O)_2OR$, $-S(O)_2R$, $-S(O)_2NR$, $-S(O)R$, $-OP(O)O_2RR$, $-P(O)O_2RR$, $-C(O)OR$, $-NR_2$, $-NR_3$, $-NC(O)R$, $-C(O)R$, $-C(O)NR_2$, –CN, and –OR, wherein R is independently selected from –H, alkyl, heteroalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl and linking group; or

R_1 taken together with R_2, Y_1, or Y_2; or

R_4 taken together with R_3, Y_3, or Y_4; or

R_5 taken together with R_6, Y_3, or Y_4; or

R_6 taken together with R_7, Y_3, or Y_4; or

R_{10} taken together with R_9 or R_{11}; or

R_{11} taken together with Y_1, or Y_2; or

R_{13} taken together with Y_3 or Y_4

are selected from alkyleno, alkyleno independently substituted with one or more Z_1, heteroalkyleno, heteroalkyleno independently substituted with one or more Z_1, aryleno, aryleno independently substituted with one or more Z_1, heteroaryleno, and heteroaryleno independently substituted with one or more Z_1;

R_8 is selected from –H, alkyl, alkyl independently substituted with one or more Z_1, heteroalkyl, heteroalkyl independently substituted with one or more Z_1, aryl, aryl independently substituted with one or more Z_1, heteroaryl, heteroaryl independently substituted with one or more Z_1, arylalkyl, arylalkyl independently substituted with one or more Z_1, heteroarylalkyl, and heteroarylalkyl independently substituted with one or more Z_1;

Y_1, Y_2, Y_3, Y_4 when taken alone are selected from –H, alkyl, alkyl independently substituted with one or more Z_1, heteroalkyl, heteroalkyl independently substituted with one or more Z_1, aryl, aryl independently substituted with one or more Z_1, heteroaryl, heteroaryl independently substituted with one or more Z_1, arylalkyl, arylalkyl independently substituted with one or more Z_1, heteroarylalkyl, and heteroarylalkyl independently substituted with one or more Z_1; or

Y_1 taken together with R_1, R_{11} or Y_2; or

Y_2 taken together with R_1, R_{11} or Y_1; or

Y_3 taken together with R_4, R_5, R_6, R_{13} or Y_4; or

Y_4 taken together with R_4, R_5, R_6, R_{13} or Y_3

are selected from alkyleno, alkyleno independently substituted with one or more Z_1, heteroalkyleno, heteroalkyleno independently substituted with one or more Z_1, aryleno, aryleno independently substituted with one or more Z_1, heteroaryleno, and heteroaryleno independently substituted with one or more Z_1; and

Z_1 is selected from –R, halogen, $-OS(O)_2OR$, $-SO_2OR$, $-SO_2R$, $-SO_2NR$, $-S(O)R$, $-OP(O)O_2RR$, $-P(O)O_2RR$, $-CO_2R$, $-NR_2$, $-NR_3$, $-NC(O)R$, $-C(O)R$, $-C(O)NR_2$, –CN, –O and –OR, wherein R is independently selected from –H, alkyl, heteroalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl and linking group.

47. The labelled nucleic acid compound of claim 46 wherein

Y_1 is taken together with R_1 or R_{11} and is C_2 or C_3 alkyleno or alkyleno independently substituted with one or more Z_1; or

Y_2 is taken together with R_1 or R_{11} and is C_2 or C_3 alkyleno or alkyleno independently substituted with one or more Z_1; or

Y_3 is taken together with R_4 or R_5 or R_6 or R_{13} and is C_2 or C_3 alkyleno or alkyleno independently substituted with one or more Z_1; or

Y_4 is taken together with R_4 or R_5 or R_6 or R_{13} and is C_2 or C_3 alkyleno or alkyleno independently substituted with one or more Z_1.

48. The labelled nucleic acid compound of claim 47 wherein the C_2 or C_3 substituted alkyleno is gem disubstituted with C_1–C_3 alkyl.

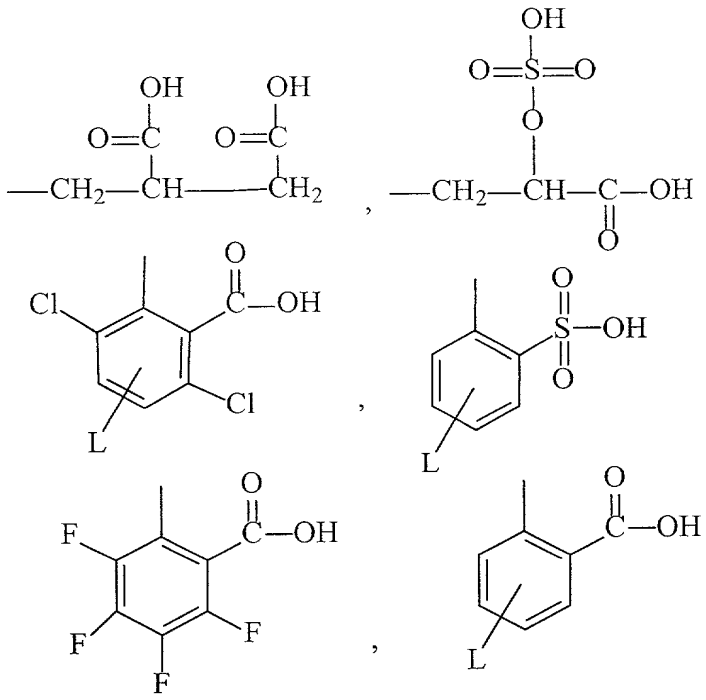
49. The labelled nucleic acid compound of claim 47 wherein the C_2 or C_3 substituted alkyleno is gem disubstituted with methyl.

50. The labelled nucleic acid compound of claim 46 wherein R_8 is alkyl independently substituted with one or more substituents selected from halogen, –C(O)R, and $-S(O)_2R$ wherein R is independently selected from–OH, O-alkyl, $-NH_2$, N-alkyl and a linkage.

51. The labelled nucleic acid compound of claim 46 wherein R_8 is $-CF_3$.

52. The labelled nucleic acid compound of claim 46 wherein R_8 is aryl or aryl independently substituted with one or more Z_1.

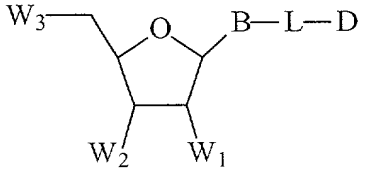
53. The labelled nucleic acid compound of claim 46 wherein R_8 is selected from the structures:



wherein L is a linkage.

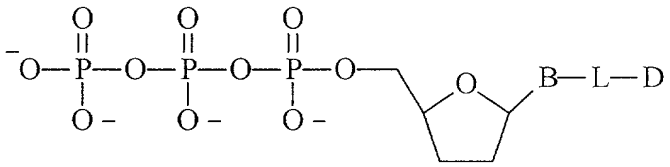
54. The labelled nucleic acid compound of claim 46 wherein NUC comprises a nucleobase selected from uracil, cytosine, deazaadenine, and deazaguanosine.

55. The labelled nucleic acid compound of claim 46 having the structure:



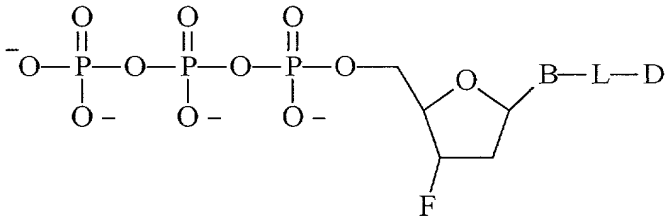
wherein B is a nucleobase; W_1 and W_2 taken separately are selected from –H, –OH, and –F; and W_3 is selected from –OH, monophosphate, diphosphate, triphosphate and phosphate analog

56. The labelled nucleic acid compound of claim 46 having the structure:



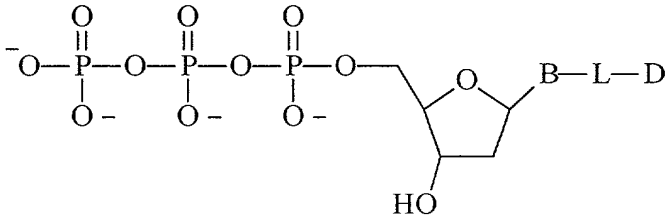
wherein B is a nucleobase.

57. The labelled nucleic acid compound of claim 46 having the structure:



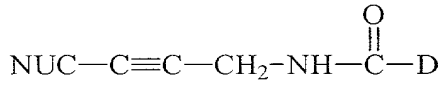
wherein B is a nucleobase.

58. The labelled nucleic acid compound of claim 46 having the structure:



wherein B is a nucleobase.

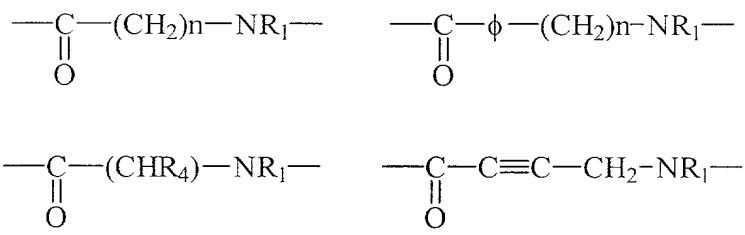
59. The labelled nucleic acid compound of claim 46 wherein L is attached to a nucleobase of NUC and to D in the structure:



60. The labelled nucleic acid compound of claim 46 wherein L is attached to a nucleobase of NUC and to D in the structure:

$$NUC—C\equiv C—CH_2OCH_2CH_2NR_3X—D$$

wherein R_3 is selected from –H and (C_1–C_6) alkyl; and X is selected from the structures:



where n ranges from 1 to 5; ϕ is aryldiyl; and R_1 is selected from –H, (C_1–C_6) alkyl and protecting group.

61. The labelled nucleic acid compound of claim 46 wherein L is attached at R_8 of D.

62. The labelled nucleic acid compound of claim 46 wherein NUC is a nucleotide and D is a donor dye and an acceptor dye wherein fluorescence energy transfer occurs between the donor dye and acceptor dye and at least one of the donor dye and acceptor dye is an extended rhodamine dye.

63. The labelled nucleic acid compound of claim 46 wherein NUC is a polynucleotide and L is attached to the polynucleotide at a position selected from the 5' terminus, the phosphodiester backbone, a nucleobase, and the 3' terminus.

64. The labelled nucleic acid compound of claim 63 wherein L is an aminohexyl linkage attached to the polynucleotide at the 5' terminus.

65. The labelled nucleic acid compound of claim 46 wherein NUC is a polynucleotide labelled with a donor dye and an acceptor dye wherein fluorescence energy transfer occurs between the donor dye and acceptor dye and at least one of the donor dye and acceptor dye is an extended rhodamine dye.

66. A method of PCR enzymatic synthesis comprising amplifying a template DNA with nucleotide triphosphates, polymerase, and two or more primers wherein the primers are complementary to the template DNA sequence and at least one of the primers is a labelled polynucleotide of claim 63.

67. A method of fragment analysis comprising the steps of:

forming one or more labeled polynucleotide fragments, the fragments being labeled with the labelled nucleic acid compound of claim 46;

resolving the one or more labeled polynucleotide fragments; and

detecting the resolved labeled polynucleotide fragments.

68. The method of claim 67 wherein the resolving step is an electrophoretic size-dependent separation process and the one or more labeled polynucleotide fragments are detected by fluorescence.

69. A kit for PCR enzymatic synthesis comprising one or more nucleotide triphosphates, polymerase, and two or more primers wherein one or more of the nucleotide triphosphates is a labelled nucleic acid compound according to claim 58.

70. A kit for PCR enzymatic synthesis comprising one or more nucleotide triphosphates, polymerase, and two or more primers wherein at least one of the primers is a labelled polynucleotide of claim 63.

71. A kit for fragment analysis comprising one or more nucleotide triphosphates, a chain-terminating nucleotide analog and a primer, wherein one or more of the nucleotide triphosphates is a labelled nucleic acid compound according to claim 55.

72. A kit for fragment analysis comprising one or more nucleotide triphosphates, a chain-terminating nucleotide analog and a primer, wherein one or more of the nucleotide triphosphates is a labelled nucleic acid compound according to claim 58.

73. A kit for fragment analysis comprising one or more nucleotide triphosphates, a chain-terminating nucleotide analog and a primer, wherein said chain-terminating nucleotide analog is a labelled nucleic acid compound according to claim 56.

74. A kit for fragment analysis comprising one or more nucleotide triphosphates, a chain-terminating nucleotide analog and a primer, wherein said chain-terminating nucleotide analog is a labelled nucleic acid compound according to claim 57.

75. A kit for fragment analysis comprising one or more nucleotide triphosphates, a chain-terminating nucleotide analog and a primer, wherein said primer is a labelled polynucleotide according to claim 63. --

II. REMARKS

Entry of this Preliminary Amendment prior to examination is respectfully requested. Obvious misspellings have been corrected by amendment in the specification. Claims 46-75 are new. Claims 1-45 are hereby cancelled.

A restriction requirement was made in Examiner's Office Action of July 19, 2000 in application Serial Number 09/325,243. New claims 46-75 correspond to, and are consonant with, Group II, claims 40-45, drawn to labeled nucleoside and nucleotides.

Support for the new claims to labelled nucleic acids can be found, for example and among other places, at: page 5, line 31 to page 6, line 6; page 13, lines 20-30; page 14, lines 12-30; page 60 line 12 to page 64, line 25. Support for the PCR method can be found at page 65, lines 6-11. Support for fragment analysis methods can be found at page 6, lines 8-11 and page 67, line 16 to page 71, line 19.. Support for labelled polynucleotides can be found at page 66, line 20 to page 67, line 7

Applicants feel that the pending claims in the present application are in condition for allowance, and allowance is therefore respectfully requested.

If any additional fees not submitted with this response are required, the Commissioner is authorized to withdraw such fees from deposit account **01-2213**.

Respectfully submitted,

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